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INTRINSIC MECHANISMS CONTROLLING CARDIAC STOMACH VOLUME OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FOLLOWING GASTRIC DISTENSION

BY DAVID J. GROVE

Marine Science Laboratories, Menai Bridge, LL59 5EY Wales, UK

AND SUSANNE HOLMGREN*

Comparative Neuroscience Unit, Department of Zoophysiology, University of Göteborg, Box 250 59, S-400 31 Göteborg, Sweden

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Summary

1. Inflation of the cardiac stomach of the rainbow trout induced reflex muscular contractions. The stomach then slowly relaxed asymptotically towards its maximum volume. Continued distension caused the stomach to become compliant and this was irreversible within the time course of each experiment (8 h). Repeated periods of rest and distension revealed a short-term inhibition of reflex contractions which recovered as resting periods were extended. Sectioning the vagosympathetic trunk did not influence the response to distension. Similar responses occurred in isolated, perfused stomachs.

2. Both tetrodotoxin and atropine plus methysergide induced immediate compliance, suggesting that it was caused by the blockade of enteric excitatory neurones. Atropine alone primarily reduced reflex contractions whilst methysergide completed this suppression and induced profound gastric relaxation.

3. Somatostatin reversibly suppressed reflex contractions whilst vasoactive intestinal polypeptide (VIP) induced gastric relaxation.

4. A model is proposed in which distension initially causes reflex activity *via* cholinergic and serotonergic nerves, whilst gastric tone remains high. Somatostatin then suppresses rhythmic contractions, whilst VIP suppresses the tryptaminergic mechanisms that maintain gastric tone.

5. The rainbow trout stomach possesses intrinsic mechanisms that mimic the extrinsic, nerve-controlled 'receptive relaxation' or 'accommodation' that follows feeding in higher vertebrates.

Introduction

In higher vertebrates, filling of the stomach leads to a reflex relaxation of the proximal (fundus and corpus) gastric musculature which is believed to be

* To whom reprint requests should be addressed.

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necessary to increase receptive capacity whilst avoiding sharp rises in intragastric pressure. The reflex was called 'receptive relaxation' by Cannon and Lieb (1911) since it can be induced by swallowing. The phenomenon depends on extrinsic nerve pathways which suppress excitatory nerves and activate vagal efferent inhibitory tracts (Martinson, 1965; Jansson, 1969; Abrahamsson, 1973; Jahnberg, 1977). Similar extrinsic reflexes can be induced by distension of the oesophagus (Jansson, 1969) or of the stomach itself (Abrahamsson and Jansson, 1973).

However, as gastric volume is deliberately increased following vagotomy, intragastric pressure rises sharply (Jansson, 1969; Staadas and Aune, 1970; Staadas, 1975). Furthermore, distension of the antrum can activate, in addition to vagal reflexes, extrinsic inhibition by way of splanchnic nerves (Abrahamsson, 1973). In addition, chemical or mechanical stimulation of the upper intestine can reflexly inhibit contraction of the stomach by vagal and/or sympathetic nerve pathways (Roman and Gonella, 1987). The term 'gastric accommodation' has been reserved for reflex, vagal relaxation induced by distension of the stomach itself, which leads to decreased activity in efferent excitatory nerves (Scratcherd and Grundy, 1982).

There is no direct evidence for similar reflex relaxation mechanisms in fish but field observations indicate that contraction of the stomach may be inhibited following ingestion of food since actively feeding fish of a variety of species have relaxed ('floppy') stomachs. The effects of vagal and splanchnic nerve stimulation on gastric muscles of teleosts have been reviewed by Nilsson (1983). Inhibitory vagal effects have been described in *Salmo gairdneri* (=*Oncorhynchus mykiss*) and *Conger conger*, whilst both inhibitory and excitatory tracts occur in *Pleuronectes platessa* and *Lophius piscatorius*. Only vagal excitatory influences were reported for *Gadus morhua* and *Platycephalus bassensis*. Splanchnic nerves contain excitatory fibres (*Salmo, Platycephalus*) or both excitatory and inhibitory tracts (*Pleuronectes*). The possibility exists that these tracts could support gastric receptive relaxation or accommodation in fish, but no experiments have been reported.

The present study has, therefore, been undertaken to examine gastric reflexes following stomach distension in the rainbow trout *Oncorhynchus mykiss* (=*Salmo gairdneri*). This species is important commercially in aquaculture but has also been the subject of relatively extensive study in relation to its feeding and gastrointestinal physiology (e.g. Grove *et al.* 1978; Holmgren and Nilsson, 1981; Holmgren, 1983; Holmgren *et al.* 1982; Holmgren *et al.* 1985; Vigna *et al.* 1985; Thorndyke and Holmgren, 1990; Aldman *et al.* 1991). It is also possible that whole-organ studies might prove useful in examining the effects of putative neurocrine and endocrine substances that have been found in the rainbow trout gastrointestinal tract. A preliminary account of the study was given in Grove (1986).

Materials and methods

Rainbow trout (600–1700 g body mass) were held in the laboratory in circulat-

ing, aerated fresh water at 12±1 °C and fed every second day with rainbow trout pellets in quantities that represented approximately 2 % of body mass per meal.

For *in situ* studies, fish (unfed for the previous 24 h) were anaesthetized in MS 222 (1:20 000) and held ventral-side-up on an operating table whilst the gills were irrigated with anaesthetic at 12–13.5 °C. A balloon (approximately 100 ml maximum volume) was passed down the oesophagus into the cardiac part of the stomach. The balloon was connected by a water-filled tube (6 mm i.d.) to a wide reservoir (capacity 100 ml), suspended from a Grass FT 03C strain gauge. Clamping the tube to the movable mounting allowed the reservoir to be raised or lowered whilst the volume of water remaining in the reservoir was recorded on a Grass Polygraph model 7 recorder. The dimensions were such that the reservoir could be raised from stomach level to a height of up to 20 cm, and the outflow of 20–30 ml of water into the balloon would only lower the reservoir level by a small amount (2 cm water maximum). This simple arrangement allowed both overall changes in gastric volume as well as reflex gastric contractile activity to be recorded simultaneously. During experiments, steady respiratory movements of the fish continued, indicating that the medulla oblongata was not affected by this level of anaesthesia. Fish could be held in this way for up to 12 h and subsequently recovered in the main aquarium.

In some fish, the intestinal branches of the left and right vagus were sectioned near the gills. Such fish were killed at the completion of the test without being allowed to recover from the anaesthetic. Where drugs were tested, appropriate concentrations, dissolved in 0.5–1 ml of saline, were injected into the caudal vein.

For *in vitro* studies, the stomach was perfused through the coeliac artery with rainbow trout Ringer and drained by the hepatic portal vein (Holmgren *et al.* 1985). The organ was removed and mounted in an organ bath so that external nerve reflexes were abolished. Test drugs (0.1–1 μmol l^{-1} in rainbow trout Ringer) were infused into the arterial catheter to determine any effects on the responses to gastric distension. At least 20 min exposure to any drug was allowed prior to testing its effect on distension.

Calculations

When describing and quantifying the responses collected using this distension technique, we have adopted the following terms (indicated in Fig. 1). (1) With the reservoir at stomach height (i.e. the applied hydrostatic pressure set at zero), no water enters the intragastric balloon and the reservoir holds its maximum water mass. The uninflated balloon and intragastric tubing occupied approximately 4 ml. (2) On raising the reservoir to apply a stated hydrostatic pressure, rapid entry of water from the reservoir into the balloon partially distended the stomach (phase 1). This volume (V_{res}) equals the change (decrease) in reservoir mass (which is recorded) and is usually limited by a reflex contraction of the stomach. The water inflow rate in this phase is close to that imposed by the resistance of the balloon itself to distension. (3) Following phase 1, in a stomach subjected to its first distension, a prolonged and slow further increase in gastric volume develops

(phase 2). This is typically accompanied by the development of spontaneous contractions of the smooth muscles of the cardiac stomach wall. This expansion (relaxation) occurs at a steadily decreasing rate until volume becomes maximal (V_{max}).

In the analysis that follows, changes in gastric volume (such as changes in the size of V_{res} with time, or relaxation rates from V_{res} to V_{max}) are treated as exponential rates of change. For example, in phase 2, the water that finally enters the stomach is $V_{max}-V_{res}$ and, at any time (t, min) in phase 2 before this phase is completed, stomach volume (V, ml) is given by the equation:

$$V = V_{res} + V_t ,$$

where

$$V_t = (V_{max} - V_{res})(1 - \exp^{-Kt}) .$$

Thus, the gastric filling found in phase 2 develops at an instantaneous rate $K\,min^{-1}$. Changes in the ability of the stomach to distend when subjected to a given pressure can be recorded as changes in the variables V_{res}, V_{max} and/or K.

Drugs

The drugs used in the present study were atropine sulphate (Sigma); phentolamine hydrochloride (Sigma); DL-propranolol hydrochloride (Sigma); MS 222 (tricaine methane sulphonate, Sigma); methysergide (1-methyl-lysergic acid butanolamine bimaleate, Sandoz); synthetic somatostatin-14 (Sigma); tetrodotoxin (TTX, Sigma); natural porcine vasoactive intestinal polypeptide (VIP, V. Mutt, Stockholm).

Results

Pattern of response to a first distension in situ *and* in vitro

Gastric distension was achieved by raising the fluid-filled reservoir to a known height above its attached intragastric balloon. The reservoir content was measured continuously. Pressures in excess of 0.2 kPa were required to induce clear responses. For a 630 g fish stimulated at high pressure (1.2 kPa), an immediate entry of 4–5 ml ($=V_{res}$) of fluid occurred, followed by the onset of spontaneous contractions and a slow, subsequent increase in cardiac gastric volume (Fig. 1). The maximum volume accepted (V_{max}) tended to increase irregularly with fish size. Values were between 10 and 30 ml over the size range 600–1900 g body mass.

Six fish of similar size (600–750 g) were tested using this method, with the following results for the first occasion when distension was induced. In phase 1, V_{res} increased with pressure at approximately 6–7 ml kPa^{-1} up to a maximum of 5–8 ml. In phase 2, relaxation occurred at an instantaneous rate of 0.03–0.05 min^{-1}, i.e. half of the distension from V_{res} to V_{max} (an entry of a further 2.75 ml) required 15–20 min.

If a newly distended stomach is deflated and then reinflated, fluid enters the relaxed stomach against a decreased resistance and V_{res} increases with each subsequent trial (Fig. 2B).

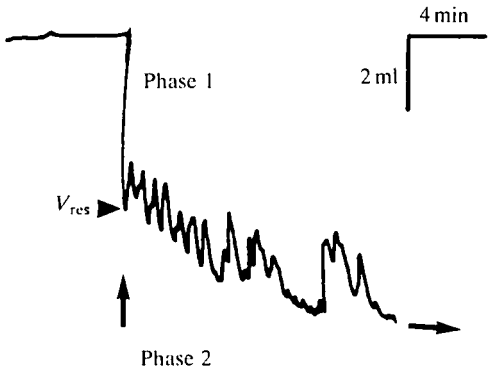

Fig. 1. Efflux of water from the reservoir to the intragastric balloon in an anaesthetized rainbow trout. At the vertical arrow, the reservoir was raised above the stomach for the first time, giving a hydrostatic pressure of 1.2 kPa. An immediate inflow of water to the balloon occurred (phase 1, volume=V_{res}) followed by a slow exponential relaxation with time towards maximum volume (V_{max}) at an instantaneous rate $K\,min^{-1}$ (phase 2). During this last phase, rhythmic muscular contractions occurred. In this and subsequent figures, the horizontal bar represents time and the vertical bar is volume. Temperature is 13 °C.

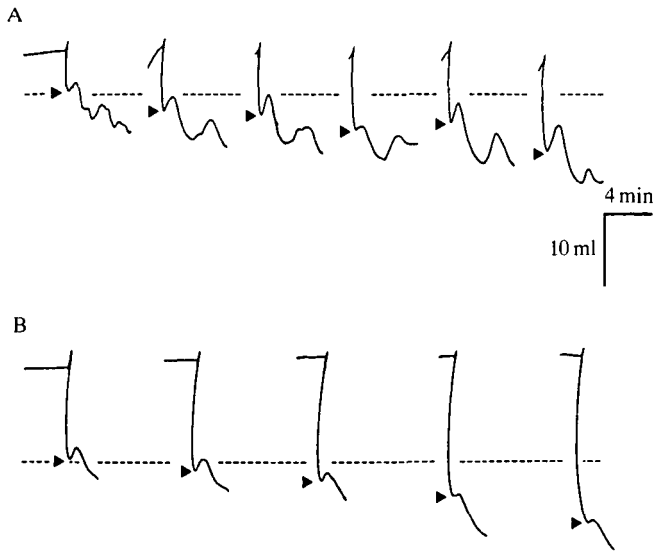

Fig. 2. Examples of the effect of repeated, brief distensions on the magnitude of V_{res} *in vitro* (A) or *in situ* (B). V_{res} increases with time whether the hydrostatic pressure is removed for short (2 min, A) or longer (20 min, B) periods between tests. Arrowheads indicate V_{res}, broken line indicates V_{res} on first distension. A, applied hydrostatic pressure on each distension 0.6 kPa, 11 °C; B, 0.7 kPa, 12 °C.

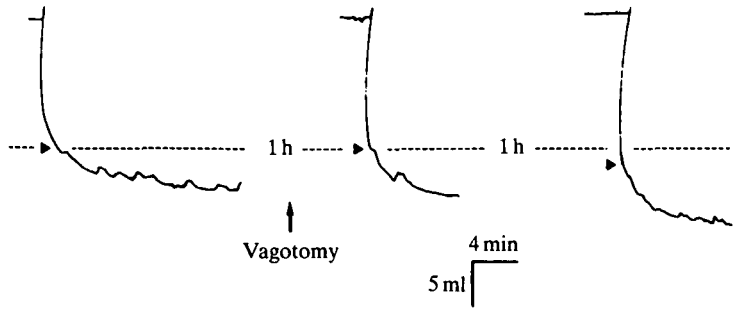

Fig. 3. Effect of bilateral section of the intestinal branches of the vagus nerve (at the arrow) on the *in situ* response to gastric distension after previous control tests. 1 h indicates 1 h of deflation between inflations. Vagotomy was performed 30 min before the second inflation. V_{res} remains large, reflex contractions stay reduced and no indication of increased gastric tone (decreased compliance) follows the operation. Arrowheads indicate V_{res}, broken line indicates V_{res} in the fully compliant stomach prior to vagotomy. Hydrostatic pressure on each distension was 1.4 kPa, 13°C.

The above observations *in situ* suggest that gastric distension using a balloon induces a slow relaxation of the gastric musculature. In mammals, this phenomenon depends on a vagal inhibitory mechanism. A preliminary test was undertaken to examine whether a similar mechanism exists in the rainbow trout. After distension, the relaxed stomachs of four rainbow trout were deflated and allowed to rest for 0.5–1 h, during which time each fish was subjected to bilateral section of the intestinal branches of the vagus nerves. These nerves are probably mixed 'vagosympathetic trunks' (Nilsson, 1983). After a further 0.5–1 h post-operative rest to ensure that the fish was respiring normally, the effects of gastric distension were again tested (Fig. 3). In all four fish, the rate of distension was rapid and undiminished; little resistance to water inflow was seen. There was no indication that inhibitory activity in the vagal nerves was essential for the maintenance of the relaxed state of the rainbow trout stomach.

Similar studies were then made on five perfused, isolated stomachs removed from rainbow trout (body mass 750–1000 g) and suspended in an organ bath. These tests were undertaken to determine responses in the absence of all extrinsic nerves, without prior distension. It was possible, as expected from mammalian studies, that the absence of inhibitory autonomic (vagal or sympathetic) pathways might limit the relaxation observed *in vitro*. In the event, no such limitation was found. Instead, the patterns of response were similar to those found *in situ* save that fluid entered the stomach with greater ease. At low pressures, V_{res} increased by 13 ml kPa^{-1}; nearly double the rate found *in situ*. Above 0.6 kPa, V_{res} increased to approximately 15–18 ml. During phase 2, the instantaneous rate of relaxation was also higher (K=0.04–0.06 min^{-1}). V_{max} was 30–45 ml in the animals tested (body mass approximately 825 g). All these measures suggest that removal from central influences does not *decrease* the ability of the cardiac stomach to relax

when distended. The pattern of relaxation seen in Fig. 1 appears to depend on changes induced in the cardiac stomach itself, and to be unaffected by extrinsic nerve reflexes. The increased distension rates seen *in vitro* could be caused by the absence of tonic (excitatory) influences of extrinsic nerves but may simply reflect removal of the stomach from mechanical constraints imposed by containment within the body wall and mesenteries.

Responses of the stomach to repeated distension

Comparison of Figs 1 and 2 shows that, once a stomach has relaxed in response to distension, it retains this relaxed state for some time, even if deflated for a period. To investigate this retained compliance, studies were made *in situ* (N=4) and *in vitro* (N=3) in which repeated distensions were made, with interposed deflations, at known time intervals. In Fig. 2A, a perfused preparation was distended for 4 min in every 6 min at low (but above-threshold) pressures. The successive re-inflations under constant conditions showed that V_{res} progressively increased. The same pattern occurred *in situ* (Fig. 2B) even when longer periods (20 min) were left between tests. We found that the stomach remained relaxed (compliant) for some time after stimulation.

In an attempt to measure the duration of such compliance, the tests shown in Fig. 4 were carried out. Fully compliant stomachs were obtained by prolonged distension (approximately 40 min) both *in situ* (N=3) and *in vitro* (N=3). When such preparations were deflated and then rapidly re-inflated, V_{res} was maximal and

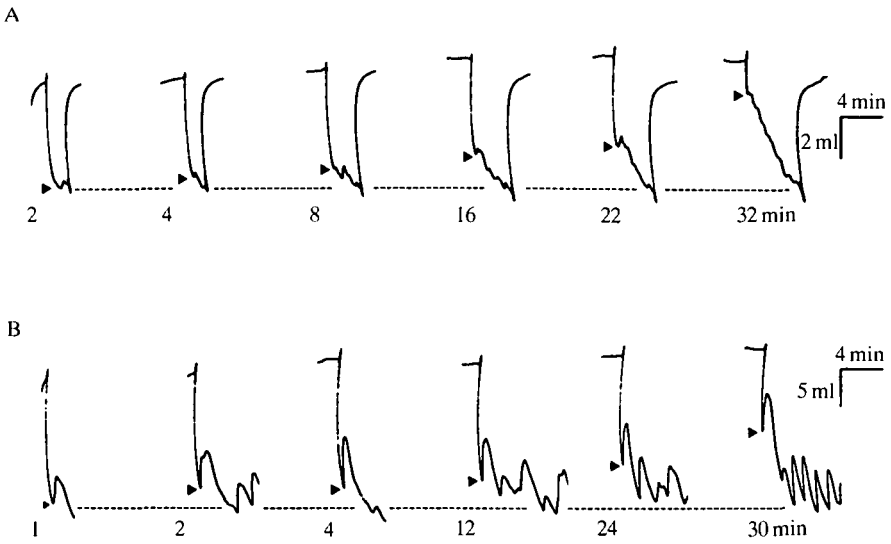

Fig. 4. Effect of increasing the duration of the rest between distensions on a previously fully compliant stomach *in situ* (A) or *in vitro* (B). V_{res} begins to decrease significantly after rest periods in excess of 15 min but relaxation rate during phase 2 remains high. Arrowheads indicate V_{res}, broken lines indicate V_{res} in the fully compliant stomach, numbers indicate the length of the rest period (min). A, hydrostatic pressure on each distension 2.0 kPa, 13°C; B, 1.0 kPa, 12°C.

approximately equal to V_{max}, i.e. there was little resistance to fluid entry. As the duration of rest periods before re-inflation increased, the magnitude of V_{res} decreased. The rate of recovery of these high values of V_{res} towards the lower, initial levels was approximately exponential, at instantaneous rates of $0.02–0.03\,min^{-1}$, indicating that half the recovery towards initial levels is completed in 20–30 min.

Close inspection of records of the type shown in Fig. 4 suggests that two mechanisms are likely to be at work. The magnitude of V_{res} (end of phase 1) is determined by the onset of reflex contractions of the gut wall in response to distension. However, when the onset of these contractions has returned to the control value (after a rest of 30 min or more between inflations), it is clear that the relaxation rate (K) during phase 2 still remains very high when compared with controls. In Fig. 5, two preparations are shown which were re-inflated after 3 h of rest. It can be seen that the relaxation rate in phase 2 is faster even after this long period of rest.

A large number of records were re-examined to find whether any pattern of change in this relaxation rate (K) could be detected with the passage of time for preparations that had been subjected to further distensions after the first test. For clarity, later values of K (K_t) are expressed as a multiple of the initial value (K_0) on first distension for that preparation. The findings are shown in Fig. 6 (with logarithmic axes for convenience of scale) and the main conclusion is that, after 20–30 min of distension, the relaxation rate during phase 2 rises steadily to reach values that typically correspond to $K=0.25–0.5\,min^{-1}$ (cf. $0.03–0.05\,min^{-1}$ at the start). In no case did we observe a reversal of this trend in K within the duration of

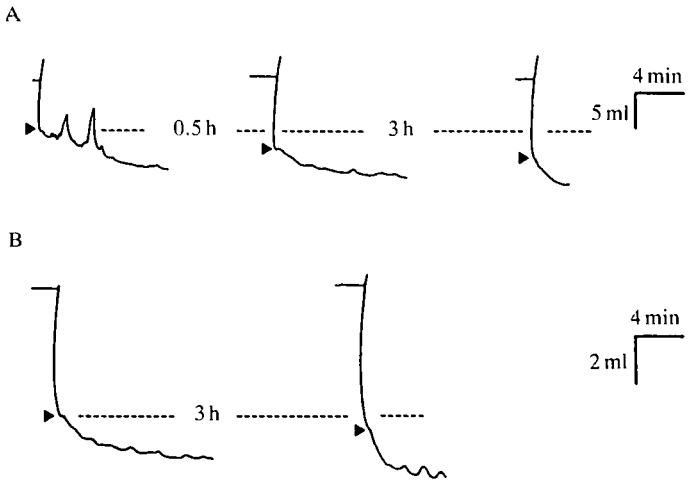

Fig. 5. Long-term increases in compliance, seen as relaxation rate during phase 2, for *in situ* (A) and *in vitro* (B) preparations of the rainbow trout stomach, following several distensions. In the last tests, both relaxation rate and V_{max} are increased when compared with the first distension. Arrowheads indicate V_{res}, broken lines indicate V_{res} on the first distension. Hydrostatic pressure on each distension was 2.9 kPa, 12 °C. 0.5 h and 3 h are the times for which the preparations were deflated between distensions.

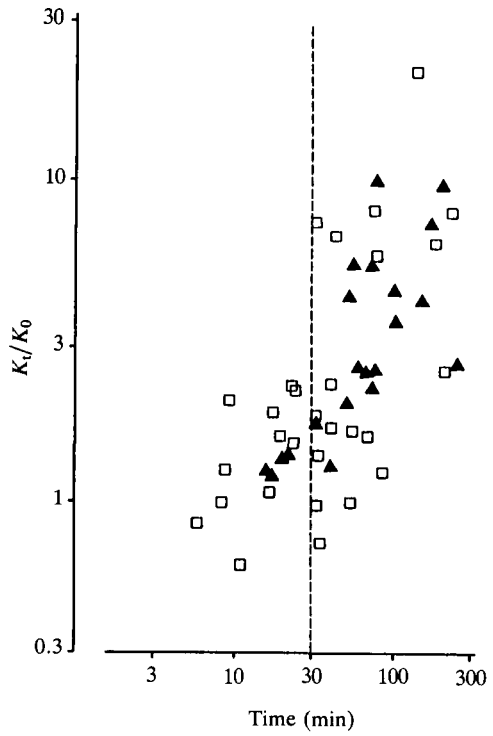

Fig. 6. The increase in relaxation rate (K) during phase 2 with time. The vertical axis represents the current rate (K_t) as a multiple of initial rate (K_0). The steady increase in compliance with time could be minimised by limiting distensions to brief periods using a distension pressure 0.4–0.6 kPa. Squares, *in vitro*; triangles, *in situ*. Note that the axes are logarithmic. Vertical dashed line represents the point at which relaxation rate starts to rise.

our experiments, which typically lasted up to 8 h and with rest periods of up to 4.5 h.

To summarise this part of the study, a stomach with an initial V_{res} of 10 ml which would distend for 15–20 min during phase 2 to reach a capacity of 30 ml would, when fully compliant, only require 2–3 min to complete phase 2. After a number of trials, we found that it was possible to delay this increase in relaxation rate (K) in phase 2 to allow sequential, comparable responses to a given inflation pressure. Low pressures (approximately 0.5 kPa) applied for a few minutes at intervals no shorter than half an hour usually gave reproducible responses, so that the effects of drugs and other agents could be tested.

Effects of drugs on responses to distension

The observations described above suggest that the stomach wall possesses its own coordinating systems that (a) induce spontaneous contractions rapidly after distension (Fig. 1); and (b) induce a slowly developing, but large, increase in the rate of relaxation of the stomach wall (Fig. 6).

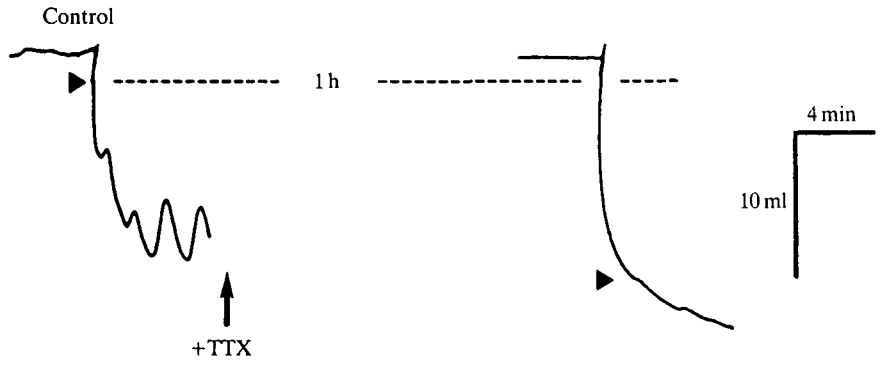

Fig. 7. Tetrodotoxin (TTX, 10^{-6} mol l^{-1}) rapidly abolishes reflex contractions of the rainbow trout stomach *in vitro*, so that relaxation rate (K) towards maximum volume (V_{max}) becomes comparable with that of a fully compliant stomach. Arrowheads indicate V_{res}, broken line indicates V_{res} on first distension, 1 h indicates time of deflation between distensions. Applied hydrostatic pressure 0.5 kPa, 11 °C.

The stomach wall of the rainbow trout is known to contain a wide range of nerves and secretory cells whose functions include the control of the smooth muscle cells. To unravel the likely control mechanisms involved in receptive relaxation, the next step was to investigate the nerve or endocrine substances involved by perfusing drugs or biochemicals into the isolated stomach by way of its arterial supply.

Fig. 7 shows the invariable response of a newly prepared stomach preparation to brief, low-pressure distension, followed by prolonged rest and perfusion with 10^{-6} mol l^{-1} tetrodotoxin (N=4). The contraction that ends phase 1 (and determines V_{res}) is abolished, subsequent spontaneous contractions disappear and the relaxation rate during phase 2 increases to 0.2 min^{-1}. Tests were carried out at earlier times in other preparations and it was found that TTX rapidly resets K to high values, comparable to those found in fully compliant stomachs. It appears that both spontaneous contractions and resistance to distension depend on activation of enteric nerve cells.

The most commonly reported excitatory neurones in the vertebrate gut release either acetylcholine (ACh) or 5-hydroxytryptamine (5-HT), and it was shown that drugs antagonistic to ACh and 5-HT affect the response of the rainbow trout stomach to distension (Fig. 8). The results shown in Fig. 8A were obtained *in situ* on a fully compliant stomach (i.e. one that had been distended several times at low hydrostatic pressure, 0.4 kPa, prior to the tests). Injection of atropine into the caudal vein suppressed the remaining weak, reflex contractions but the further injection of methysergide drastically increased relaxation rate in phase 2. Fig. 8B,C shows the effects on the responses to distension of perfusing the antagonists to 5-HT and ACh into the stomach wall *in vitro*. After the introduction of methysergide, there was an increase in V_{res}, but the contraction that ends phase 1 persisted. During phase 2, some reduction in spontaneous contractions occurred, but these were never abolished by this antagonist. Methysergide also induced an

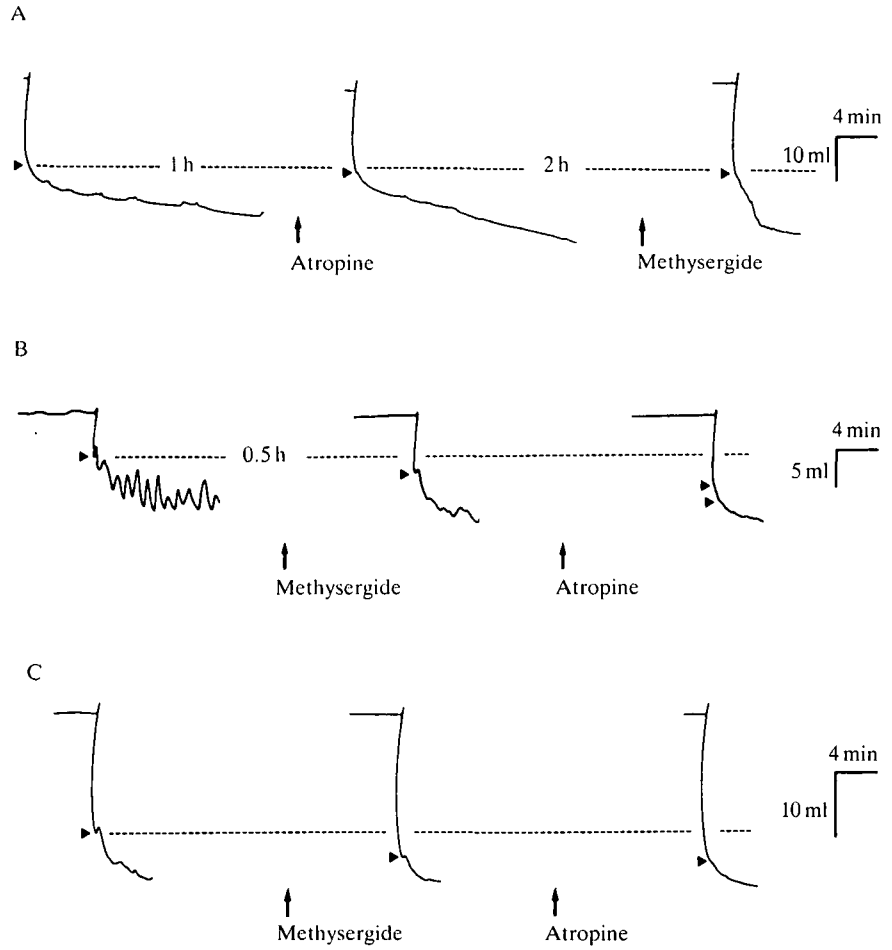

Fig. 8. (A) Atropine (1.5 μmol) injected into a compliant rainbow trout stomach *in situ* had little effect, but methysergide (1.5 μmol) immediately increased relaxation rate to high levels. (B) *In vitro* perfusion with the 5-hydroxytryptamine inhibitor methysergide (10^{-7} mol l^{-1}) in a freshly prepared stomach increases relaxation rate in phase 2, reduces reflex contractions but fails to prevent the reflex contraction that ends phase 1. Addition of atropine (10^{-7} mol l^{-1}) removes this contraction as well as residual phase 2 contractions which survive 5-hydroxytryptamine blockade. (C) The fully compliant stomach *in vitro* (subjected to higher pressure distensions) has achieved high relaxation rate and is insensitive to 10^{-6} mol l^{-1} methysergide. The reflex contraction that persists upon distension is abolished by 10^{-6} mol l^{-1} atropine. Arrowheads indicate V_{res}, broken line indicates V_{res} on first distension, 0.5 h, 1 h and 2 h indicate the durations of deflation between distensions.

increased relaxation rate during this phase as well as an immediate increase in V_{max}. Addition of atropine, however, abolished both the reflex contraction that terminated phase 1 and any residual contractions in phase 2. In four other preparations tested at low pressures, 5-HT blockade invariably increased the rate

of relaxation in phase 2 but the presence of atropine was obligatory to abolish spontaneous contractions. A fully compliant stomach distended rapidly after inflation and consequently methysergide had little effect. Atropine, however, suppressed the persisting, weak reflex contraction that terminates phase 1 (Fig. 8C).

On the assumption that intrinsic cholinergic and tryptaminergic nerves control reflex contractions and gastric muscle tone, respectively, the question remains as to which local agents could suppress their activity when the stomach fills, leading to relaxation and increased compliance. Such agents should have prolonged effects in comparison with ACh or 5-HT if the retention of compliance shown in Figs 3 and 4 is to be explained. In this study, we have concentrated on two polypeptide candidates for this role, somatostatin (SST) and vasoactive intestinal polypeptide (VIP).

The main effect of SST was to suppress both the reflex contraction that terminates phase 1 and the reflex contractions that occur in phase 2 (Fig. 9). In this regard, SST (N=3) resembles atropine, suggesting that it acts to suppress activity in cholinergic nerves that are stimulated in response to distension, without affecting maximum capacity (V_{max}).

VIP (N=4), when perfused into lightly distended preparations (Fig. 10A), in common with methysergide, partially suppressed contractile activity in phase 2

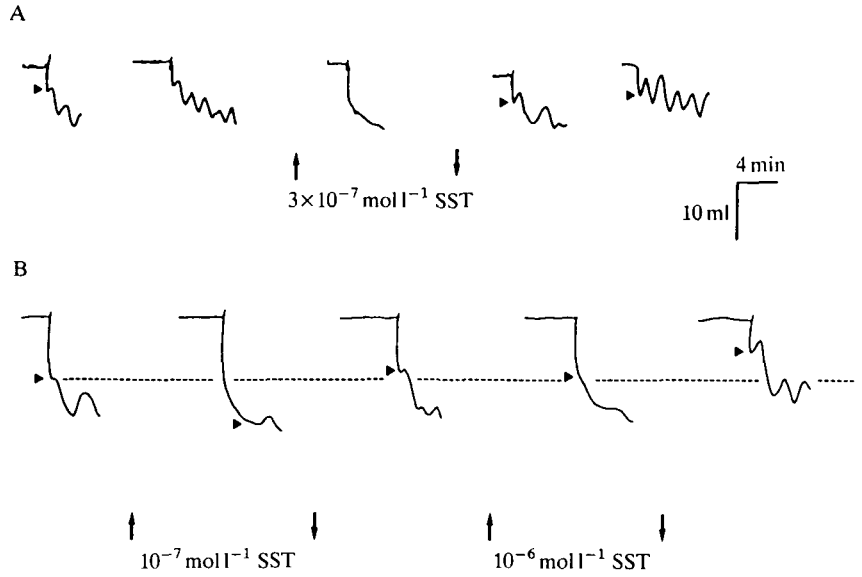

Fig. 9. During repeated inflations and deflations at low hydrostatic pressure, perfusion with somatostatin (SST) reversibly abolishes (A) or reduces (B) reflex contractions *in vitro*, including that which determines the end of phase 1, but has no effect on relaxation rate (K) in phase 2 or on V_{max}. Arrowheads indicate V_{res}, broken line indicates V_{res} on first distension. A, *in vitro*, applied hydrostatic pressure on each distension 0.5 kPa, 0.75 h delays between distensions, 12°C; B, *in vitro*, 0.6 kPa, 1.0 h delay, 11°C.

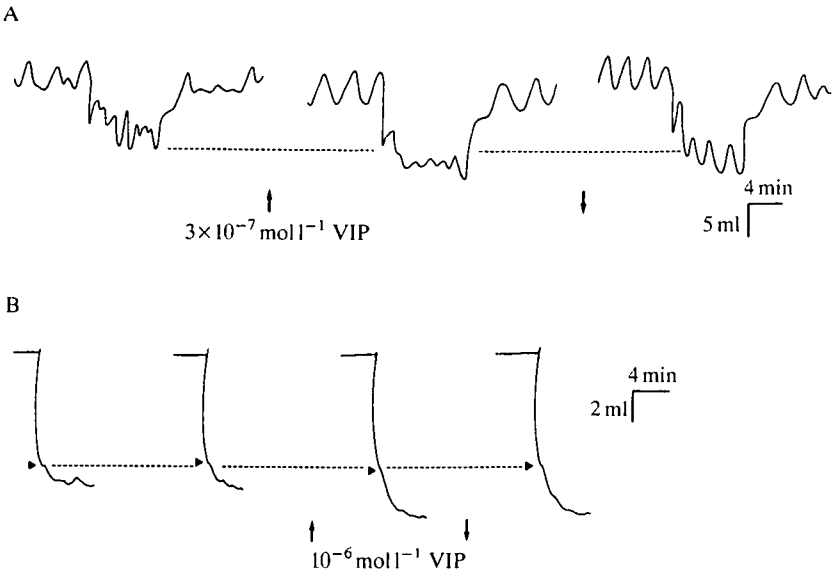

Fig. 10. Perfusion with vasoactive intestinal polypeptide (VIP) (A) reduces spontaneous contractions, increases relaxation rate in phase 2 and increases gastric volume following low-pressure distension. After washing, reflex contractions return, but relaxation rate and volume remain high. In the compliant stomach after several large distensions (B) both relaxation rate and V_{max} are increased. A, *in vitro*, distension pressure 0.5 kPa, 13 °C, broken line indicates V_{max} on first distension; B, *in vitro*, 2 kPa, 10 °C, broken line indicates V_{res} on first distension.

and increased both K and V_{max}. It also failed to abolish the contraction that terminates phase 1. When VIP was removed from the perfusate, spontaneous activity in phase 2 rapidly recovered, but the increase in both K and V_{max} persisted, even after delays of more than 1 h. In more strongly distended stomachs (Fig. 10B), where compliance was high, VIP noticeably increased V_{max} and this effect persisted for at least 1 h after washing through with Ringer.

Discussion

In common with other salmonids, the rainbow trout has a well-developed stomach that is divided into a receptive, cardiac region leading into a narrower, muscular pyloric region which coordinates transfer of food to the intestine *via* the pyloric sphincter. When feeding voluntarily on suitably sized particles, it is not unusual for a hungry rainbow trout of the size used here to consume meals approaching 2.5 % of its body mass. However, satiation times of rainbow trout well in excess of 40 min have been recorded (Ishiwata, 1968; Grove *et al.* 1978) and this is also true of other salmonids (Brett, 1971; Elliott, 1975). The fish prefer relatively small food items, which they consume one at a time, often retiring to cover or to deeper water to handle and swallow each item before returning for another. The slow onset of full gastric relaxation in this species may be a factor in

this slow attainment of satiation. Similarly, gastric emptying requires many hours, and the prolonged relaxation that follows simple, mechanical distension is presumably an essential part of the storage and digestion process.

As a result of our experiments, we propose the following model to explain the likely sequence of mechanical changes that occur in the rainbow trout stomach as feeding begins. The acceptance of food particles during a meal leads to an increasing distension of the stomach wall. This distension is opposed primarily by reflex activation of intramural nerves, which induce muscle contractions and maintain tone. Cholinergic neurones control reflex contractions (which in our recordings terminate phase 1 and determinate V_{res}). The continuing contractions (seen in phase 2 of our study) probably correspond with existing gastric digestion, where the food is mixed with pepsin and hydrochloric acid in the stomach. Serotonergic nerves, whose presence has been established previously (Holmgren *et al.* 1985; Kitazawa, 1989), also help to control phase 2 contractions but, in addition, maintain the muscle tone which limits the rate of relaxation (K) towards maximum volume (V_{max}) in our study.

If gastric distension is maintained (as in feeding), the serotonergic nerve activity may be antagonized by the release of VIP, leading to a fall in tone and to profound gastric relaxation. VIP has been detected in neurones of the rainbow trout stomach (Holmgren *et al.* 1982) and, in the isolated perfused small intestine of the guinea pig, it has been found to suppress neuronal release of 5-HT following distension (Schwörer *et al.* 1989). We suggest that it is primarily this long-term suppression of serotonergic stimulation by VIP that is responsible for the high level of compliance which develops in the distended stomach (see Fig. 6).

The possible role of somatostatin in the physiological response to feeding is less clear. Somatostatin may be released from endocrine cells of the stomach wall or from more posterior regions of the gastrointestinal tract and its associated glands as chyme leaves the stomach and enters the intestine. Endocrine cells showing somatostatin-like immunoreactivity are present in the mucosa of the stomach of the rainbow trout (Holmgren *et al.* 1982), and somatostatin cells frequently occur in pancreatic tissues of salmonid species (Wang *et al.* 1986; Nozaki *et al.* 1988). The short-term suppression by somatostatin of enteric nerves that induce contraction in phase 2 would serve as a mechanism to control gastric emptying. Inhibition of the stomach in the rainbow trout may, therefore, include long-term, VIP-induced receptive relaxation, to accommodate a meal, as well as shorter-term, SST-induced inhibition of contractions necessary to control pulsatile gastric emptying.

It must be emphasised that many other factors, as yet unknown, may also be involved in the control of gastric motility in the rainbow trout, since new types of nerves and endocrine cells continue to be detected. The composition of the food is known to affect the motility and gastric emptying. For example, Grove *et al.* (1978) have shown for the rainbow trout that an increase in dietary energy slows gastric emptying (and consequently limits food intake) by increasing the time between voluntarily ingested meals. However, little is known of the mechanisms of action and which neurones and endocrine cells are mediating the effect.

Furthermore, it cannot be claimed that extrinsic nerves, such as the vagus or sympathetic, have no role to play. The present *in situ* study used only anaesthetized fish, and the actions of the anaesthetic on central reflexes, if any, are as yet unknown.

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References

ABRAHAMSSON, H. (1973). Studies on the inhibitory nervous control of gastric motility. *Acta Physiol. Scand.* (Suppl.) **390**, 1–38.

ABRAHAMSSON, H. AND JANSSON, G. (1973). Vago-vagal gastro-gastric relaxation in the cat. *Acta physiol. scand.* **88**, 289–295.

ALDMAN, G., GROVE, D. J. AND HOLMGREN, S. (1991). Duodenal acidification and intra-arterial injection of CCK8 increase gallbladder motility in the rainbow trout *Oncorhynchus mykiss*. *Gen. comp. Endocr.* (in press).

BRETT, J. (1971). Satiation time, appetite and maximum food intake of sockeye salmon (*Oncorhynchus nerka*). *J. Fish. Res. Bd Can.* **28**, 409–415.

CANNON, W. B. AND LIEB, C. W. (1911). The receptive relaxation of the stomach. *Am. J. Physiol.* **29**, 267–273.

ELLIOTT, J. M. (1975). Weight of food and time to satiate brown trout (*Salmo trutta* L). *Freshwater Biol.* **5**, 51–64.

GROVE, D. J. (1986). Gastrointestinal physiology: rate of food processing in fish. In *Fish Physiology: Recent Advances* (ed. S. Nilsson and S. Holmgren), pp. 140–152. London: Croom Helm.

GROVE, D. J., LOIZIDES, L. AND NOTT, J. (1978). Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. *J. Fish Biol.* **12**, 507–516.

HOLMGREN, S. (1983). The effect of putative non-adrenergic, non-cholinergic autonomic transmitters on isolated strips from the stomach of the rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* **74C**, 229–238.

HOLMGREN, S., GROVE, D. J. AND NILSSON, S. (1985). Substance P acts by releasing 5-hydroxytryptamine from enteric neurons in the stomach of the rainbow trout, *Salmo gairdneri*. *Neuroscience* **14**, 683–693.

HOLMGREN, S. AND NILSSON, S. (1981). On the non-adrenergic, non-cholinergic innervation of the rainbow trout stomach. *Comp. Biochem. Physiol.* **70C**, 65–69.

HOLMGREN, S., VAILLANT, C. AND DIMALINE, R. (1982). VIP-, substance P-, gastrin/CCK-, bombesin-, somatostatin-, and glucagon-like immunoreactivities in the gut of the rainbow trout, *Salmo gairdneri*. *Cell Tissue Res.* **223**, 141–153.

ISHIWATA, N. (1968). Ecological studies on the feeding of fishes. Frequency of feeding and satiation amount. *Bull. Jap. Soc. Sci. Fish.* **36**, 979–984.

JAHNBERG, J. (1977). Gastric adaptive relaxation. Effects of vagal activation and vagotomy. An experimental study in the cat. *Scand. J. Gastroenterol.* (Suppl.) **46**, 5–32.

JANSSON, G. (1969). Extrinsic nervous control of gastric motility. An experimental study in the cat. *Acta physiol. scand.* (Suppl.) **326**, 1–42.

KITAZAWA, T. (1989). 5-Hydroxytryptamine is a possible neurotransmitter of the non-cholinergic excitatory neurones in the longitudinal muscle of the rainbow trout stomach (*Salmo gairdneri*). *Br. J. Pharmac.* **98**, 781–790.

MARTINSON, J. (1965). Vagal relaxation of the stomach. Experimental re-investigation of the concept of the transmission mechanism. *Acta physiol. scand.* **64**, 453–462.

NILSSON, S. (1983). *Autonomic Nerve Function in the Vertebrates*. Berlin, Heidelberg, New York: Springer Verlag.

NOZAKI, M., MIYATA, K., OOTA, Y., GORBMAN, A. AND PLISETSKAYA, E. M. (1988). Different

cellular distributions of two somatostatins in brain and pancreas of salmonids, and their separate associations with insulin and glucagon secretion cells. *Gen. comp. Endocr.* **69**, 267–280.

ROMAN, C. AND GONELLA, J. (1987). Extrinsic control of digestive tract motility. In *Physiology of the Gastrointestinal Tract* (ed. L. R. Johnson), pp. 507–553. New York: Raven Press.

SCHWÖRER, H., RACKE, K. AND KILBINGER, H. (1989). Effect of vasoactive intestinal polypeptide on the release of serotonin from the *in vitro* vascularly perfused small intestine of guinea-pig. *Naunyn-Schmiedeberg's Arch. Pharmac.* **339**, 540–545.

SCRATCHERD, T. AND GRUNDY, D. (1982). Nervous afferents from the upper gastrointestinal tract which influence gastrointestinal motility. In *Motility of the Digestive Tract* (ed. M. Weinbeck), pp. 7–17. New York: Raven Press.

STAADAS, J. O. (1975). Intragastric pressure/volume relationship before and after proximal gastric vagotomy. *Scand. J. Gastroenterol.* **10**, 129–134.

STAADAS, J. O. AND AUNE, S. (1970). Intragastric pressure/volume relationship before and after vagotomy. *Acta chir. scand.* **136**, 611–615.

THORNDYKE, M. AND HOLMGREN, S. (1990). Bombesin potentiates the effect of acetylcholine on isolated strips of fish stomach. *Regulatory Peptides* **30**, 125–135.

VIGNA, S. R., FISHER, B. L., MORGAN, J. L. M. AND ROSENQUIST, G. L. (1985). Distribution and molecular heterogeneity of cholecystokinin-like immunoractive peptides in the brain and gut of the rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* **82**C, 143–146.

WANG, Y. Q., PLISETSKAYA, E., BASKIN, D. G. AND GORBMAN, A. (1986). Immunocytochemical study of the pancreatic islets of the Pacific salmon, *Oncorhynchus kisutch*. *Zool. Sci.* **3**, 123–129.